Synthesis and Pharmacological Evaluation of Novel Triazolo [4, 3-*a*] tetrahydrobenzo (*b*) thieno [3, 2-*e*] pyrimidine-5(4*H*)-ones

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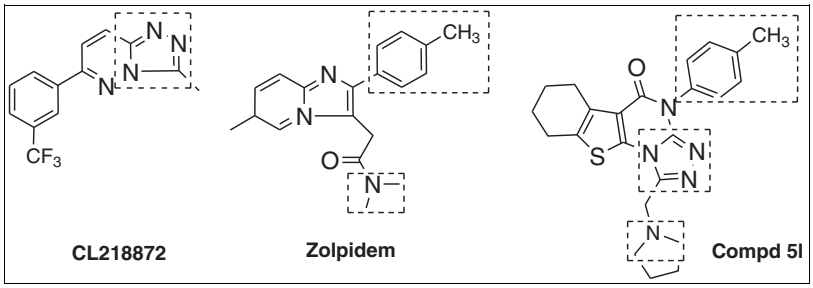

Triazolo[4,3-*a*]tetrahydrobenzo(*b*)thieno[3,2-*e*]pyrimidine-5(4*H*)-ones (**5a–m**) were synthesized and characterized by spectral analysis. All 13 derivatives were evaluated for central nervous system (CNS) depressant and skeletal muscle relaxant activities in Swiss albino mice. All the activities were compared with diazepam as a standard drug at a dose of 5 mg/kg. The most active derivatives **5d**, **5e**, **5k**, and **5l** in CNS depressant and skeletal muscle relaxant activities were selected for anticonvulsant activity. The active derivatives were found to protect 100% mice at the dose of 6–11 mg/kg, where as standard diazepam protect the animal at a dose of 2.5 mg/kg.

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INTRODUCTION

Chronic anxiety and epilepsy are common and serious disorder of central nervous system (CNS). Anxiety is even more common with a lifetime prevalence of 5% for generalized anxiety disorder. Although the genesis and appearance of anxiety differ from epilepsy but both can be treated by modulation of $GABA_A$ receptor [1]. Central nervous system (CNS) depressant agents are an important class of drugs, which are useful in the treatment of anxiety and related emotional disorders. Among the different classes of CNS depressant agents, benzodiazepines have good activity and well accepted by patients. They are acting through benzodiazepine receptors, which are adjacent to major inhibitory neurotransmitter γ-amino butyric acid (GABA) receptors in the brain and controls excitability of many CNS pathways. GABA exerts its physiological effects by binding to the different receptor types in the neuronal membrane: $GABA_A$, $GABA_B$, and $GABA_C$ receptors. The $GABA_B$ receptor belongs to the G-protein coupled receptors super family, whereas the $GABA_A$ [2] and $GABA_C$ [3] are ligand-gated chloride ion channel complex. $GABA_A$ receptors are responsible for the majority of neuronal inhibition in the mammalian CNS and mediate the action of many pharmacological useful agents viz. benzodiazepines, barbiturates, neuroactive steroids, anesthetics, and convulsants [4,5]. At least two classes of compounds have been identified by their ability to modulate GABA neurotransmission by interacting with receptor complex. GABA neurotransmission that leads to an increase in GABA-induced chloride is modulated by benzodiazepine and non-benzodiazepine agents. Among the non-benzodiazepine agent's zopiclone [6], CL 218872 and zolpidem [7] are found useful (Fig. 1).

Some non-benzodiazepine ligands are apparently selective for $GABA_A$ receptors, which have reduced sedation than benzodiazepine. All these non benzodiazepine ligands were found to contain common polyaza system. The condensed triazole and 1, 2, 4-triazole are also found to contain polyaza ring system.

The literature survey reveals that triazoles were reported for analgesic, anti-inflammatory, anti-allergic, and CNS depressant activities. Number of references [8–13] showed that condensed 1, 2, 4-triazoles exhibited excellent CNS depressant and anticonvulsant activities. Significant CNS depressant activity was reported for triazoles, especially triazoloquinazoline [14], triazoloquinazolinediones [15], triazolopyrimidines [16], triazolothienopyrimidine [17], 1,3,4-thiadiazolotetrahydrobenzothienopyrimidine [18], and 1,3,4-thiadiazole-quinazoline [19] have given an impetus to synthesize some non-benzodiazepine ligands (bioisosteric triazolotetrahydrobenzo(*b*)thienopyrimidines), which are devoid of typical benzodiazepine mediated side effect such as physical dependence, amnesia, and over sedation.

As a part of our ongoing medicinal chemistry research program, we have documented that 1-substituted triazolo [4,3-*a*]tetrahydrobenzo(*b*)thieno[3,2-*e*]pyrimidine-5(4*H*)-ones [20] exhibited good CNS depressant, skeletal muscle

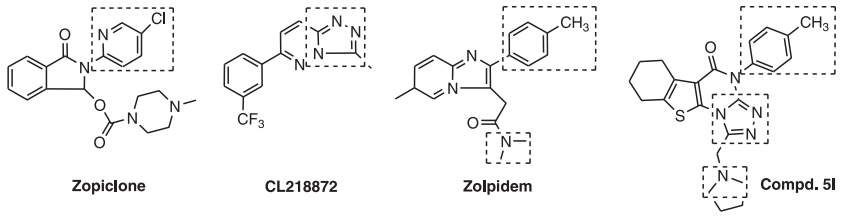


Figure 1. Structures of non-benzodiazepine ligands and most active compound (5l).

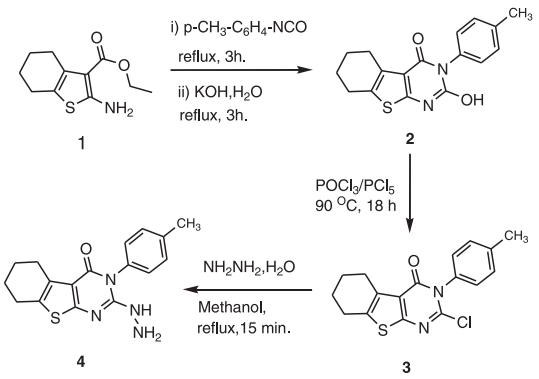
relaxant, and anticonvulsant activities. The present work is an extension of our ongoing efforts towards the development and identification of new molecules for better CNS activity. In our previous study [20], we have reported the effect of electron withdrawing group chlorine on the biological activity. But as zolpidem possess the electron donating group methyl substituent in the aromatic ring, we thought to replace chloro group with methyl group in our targeted structure with the motto to change the electronic character of a molecule and study its effects on biological activity. On this fact, we have synthesized a series of 13 novel derivatives of triazolo[4,3-*a*]tetrahydrobenzo(*b*)thieno[3,2-*e*] pyrimidine-5(4*H*)-ones. The synthesized compounds were tested for CNS depressant, skeletal muscle relaxant, and anticonvulsant activities.

RESULTS AND DISCUSSION

Chemistry. The key intermediate 2-hydroxy-3-(4-methylphenyl)-5,6,7,8-tetrahydrobenzo(b)thiophene[2,3-*d*] pyrimidine-4(3*H*)-one (**2**) was prepared by refluxing 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(*b*) thiophene [21] (**1**) with solution of *p*-tolylisocyanate [22] at 90°C in dichloroethane followed by cyclization with potassium hydroxide solution. The compound (**2**) on treatment with mixture of phosphorus oxychloride and phosphoruspentachloride yielded the 2-chloro-3-(4-methylphenyl)-5,6,7,8-tetrahydrobenzo(*b*)thiophene[2,3-*d*] pyrimidine-4(3*H*)-one (**3**) at refluxing condition. The chloro derivative (**3**) on treatment with hydrazine hydrate in methanol yielded 3-(4-chlorophenyl)-2-hydrazino-5,6,7,8-tetrahydrobenzo (b)thiophene[2,3-*d*]pyrimidine-4(3*H*)–one (**4**) as shown in Scheme 1. The title compounds (**5a–j**) were obtained in fair to good yield through the cyclization of hydrazino derivative (**4**) with one carbon donors such as triethyl orthoformate, triethyl orthoacetate, propionic acid, butyric acid, isobutyric acid, chloroacetyl chloride, cyanogens bromide, methyl isothiocyanate and carbon disulphide at reflux temperature [23–28].

Compounds (**5k–m**) were obtained by nucleophilic displacement of chlorine of compound **5j** with various alicyclic amines such as pyrrolidine, piperidine, and morpholine. The title compounds (**5a–m**) were synthesized by the route depicted in Scheme 2. The IR spectra of these

Scheme 1. Synthesis of hydrazino intermediate (4).



compounds showed intense peaks at1675–1685 cm^{-1} for carbonyl (C═O) stretching. The formation of **4** was confirmed by the presence of NH and NH_2 signals around 3340–3225 cm^{-1} in the IR spectra. It also showed a peak for carbonyl (C═O) at around 1685 cm^{-1}. The NMR spectrum of the compound **4** showed signals at δ 5.0 (bs, 2H, NHNH_2), and 8.7 (bs, 1H, N*H*NH_2). The formation of cyclic product is indicated by the disappearance of peaks due to NH and NH_2 of the starting material at 3400–3200 cm^{-1} in IR spectra of all the compounds (**5a–m**). The NMR spectrum of (**5a–m**) showed the absence of NH and NH_2 signals. A two doublet signals at 7.25–7.60 integrating for aromatic protons were observed. The mass spectra of the synthesized final compounds gave molecular ion peak exactly at M + 1 indicated that the data are in agreement with the predicted structures. The structures of these new compounds were confirmed by elemental and spectral analysis.

Pharmacological activity. The title derivatives (**5a–m**) were evaluated for CNS depressant activity and skeletal muscle relaxant activity (Motor coordination) at a dose of 5 mg/kg in Swiss albino mice. The activity was compared with diazepam as a standard drug. Of the various compounds tested for CNS depressant and skeletal muscle relaxant activities, the most active four derivatives **5d**, **5e**, **5k**, and **5l** were evaluated for anticonvulsant activity at different dose levels. The results of the various activities are presented in Tables 1, 2, 3, 4, and 5.

All the derivatives were tested for CNS depressant activity by photoactomer. The decrease in the locomotor

Scheme 2. Synthesis of target compounds (5a–m).

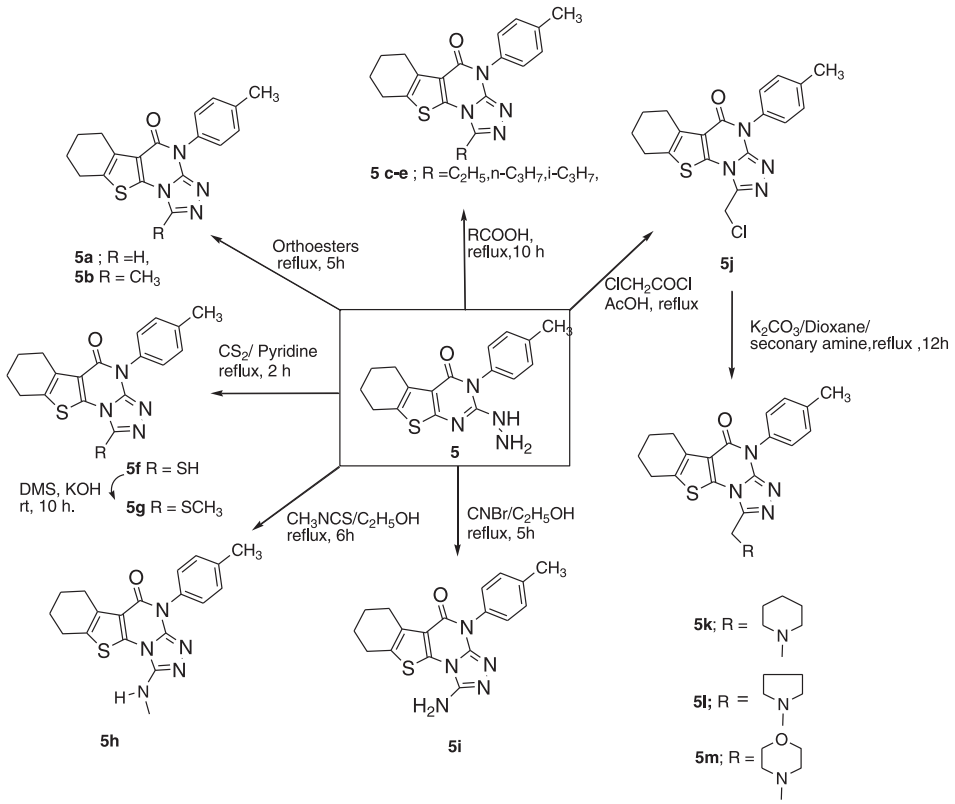


Table 1

CNS Depressant activity by Photoactometer ($n = 6$ animals; dose 5 mg/kg i.p.).

Compound	Photoactometer counts[a]		%CNS depressant activity	
	30 min after	60 min after		
	Prior(control)	Administration of test compound	After 30 min	After 60 min
5a	96 ± 1.74	25 ± 1.28	20 ± 1.28	73.95	79.16
5b	110 ± 1.78	31 ± 1.44	19 ± 1.25	71.81	82.72
5c	112 ± 1.83	30 ± 1.44	19 ± 1.28	73.21	83.03
5d	115 ± 1.77	22 ± 1.23	15 ± 1.17	80.86	86.95
5e	123 ± 1.67	22 ± 1.44	14 ± 1.24	82.11	88.61
5f	114 ± 1.73	30 ± 1.20	25 ± 1.24	73.68	78.07
5g	118 ± 1.74	32 ± 1.40	21 ± 1.26	72.88	82.20
5h	138 ± 1.69	42 ± 1.48	35 ± 1.32	69.56	74.63
5i	107 ± 1.70	37 ± 1.22	12 ± 1.13	65.42	70.09
5j	135 ± 1.76	34 ± 1.47	25 ± 1.25	74.81	81.48
5k	120 ± 1.75	17 ± 1.15	12 ± 1.12	85.83	90.00
5l	111 ± 1.68	14 ± 1.11	10 ± 1.10	87.38	90.99
5m	125 ± 1.71	30 ± 1.34	21 ± 1.30	76.00	83.20
Diazepam	102 ± 1.60	14 ± 1.21	10 ± 1.17	86.27	90.19

CNS, central nervous system.
[a]Each score represents the means ± SEM of six mice, significantly different from the control score at $p < 0.05$ (Student's *t*-test).

activity indicates the CNS depressant activity. All compounds showed the decrease in locomotor activity between 70.0 to 91.0% after 60 min, when compared with diazepam as reported (Table 1). The four derivatives **5d**, **5e**, **5k**, and **5l** showed comparable CNS depressant activity at a dose of 5 mg/kg *i. p.* after 60 min of administration to that of

standard drug diazepam (90.0%) at a dose of 5 mg/kg. Compounds **5k** and **5l** were equipotent to diazepam in decreasing % locomotor activity. All the compounds except **5a**, **5f**, **5h**, and **5i** exhibited more than 80% decrease in locomotor activity after 60 min. In a similar study using swim pool test, the immobility time after administration of the test compounds was compared with diazepam (Table 2). Except for **5a**, **5f**, and **5i**, other tested compounds were found to exhibit potent CNS depressant activity as indicated by increased immobility time.

The title derivatives were also tested for skeletal muscle relaxant activity (Motor coordination) by rotarod method (Table 3). In this model, the four derivatives **5d**, **5e**, **5k**, and **5l** showed the better activity in the range from 101.0 to 107.0% when compared to diazepam (100%) at a dose of 5 mg/kg. The other derivative showed the activity in the range of 63.0 to 93.0% to that of standard drug. The most active four derivatives **5d**, **5e**, **5k**, and **5l** among the series were evaluated for anticonvulsant activity (Table 4). The dose required for protecting the animal from pentylenetetrazole (PTZ) induced clonic convulsions was determined. All the four compounds offered good protection to the animals from the PTZ induced clonic convulsions at the dose levels of 6.0–11.0 mg/kg body weight, whereas diazepam shown 100% protection at the dose of 2.5 mg/kg. Again, the compound **5l** exhibited the anticonvulsant activity by protecting 100% animal at dose of 6.0 mg/kg body weight. The ED_{50} values for anticonvulsant activity of four compounds have also been calculated and found in the range of 4.50 to 9.50 mg/kg, whereas that of diazepam showed the ED_{50} 1.0 (Table 5).

The pharmacological results indicated that the series of compounds exhibited comparable CNS depressant and skeletal muscle relaxant than anticonvulsant activities. 1-Propyl, isopropyl, pyrrolidinemethyl, piperidinemethyl, and morpholinomethyl substituted compounds **5d**, **5e**, **5k**, **5l**, and **5m** possessed better CNS activities as compared

Table 2

CNS study on triazolo[4, 3-*a*]tetrahydrobenzo(*b*) thieno[3, 2-*e*] pyrimidine-5(4*H*)-one derivatives for CNS depressant by Forced Swim Pool test.

	Immobility time[b]	
Compound[a]	Control (24 h prior)	Post treatment (60 min after)
5a	114.33 ± 7.56	124.56 ± 10.89
5b	54.10 ± 13.71	90.45 ± 13.65
5c	96.12 ± 6.22	145.37 ± 10.11
5d	63.56 ± 12.44	118 ± 12.11
5e	120.45 ± 11.55	190.34 ± 10.67
5f	65.67 ± 7.98	70.65 ± 8.76
5g	101.56 ± 10.43	127.65 ± 8.87
5h	78.43 ± 9.69	98.23 ± 10.54
5i	49.54 ± 12.22	60.48 ± 12.96
5j	133.49 ± 8.34	176.48 ± 9.61
5k	125.41 ± 10.22	201.98 ± 11.33
5l	134.77 ± 11.65	221.88 ± 12.35
5m	105.20 ± 8.76	199.54 ± 9.39
Diazepam[a]	120.44 ± 14.29	233.29 ± 15.03

CNS, central nervous system.
[a]The compounds and diazepam were tested at a dose of 5 mg/kg (*i p.*). Each group consisted of six mice.
[b]Each value represents the means ± SEM of six mice, significantly different from the control value at $p < 0.05$ (Student's *t*-test).

Table 3

Skeletal muscle relaxant activity (Motor coordination) by Rotarod method ($n = 6$ animals; dose 5 mg/kg, i.p.).

Compound	Mean number of falls/min (Control)[a]	Mean increase in number of falls/min[a]	% CNS depressant activity
5a	2.86 ± 0.22	6.25 ± 0.52	73.52
5b	2.82 ± 0.34	6.40 ± 0.71	78.74
5c	2.70 ± 0.33	6.75 ± 0.30	93.04
5d	2.44 ± 0.35	6.40 ± 0.61	100.67
5e	2.83 ± 0.19	7.45 ± 0.38	101.26
5f	2.90 ± 0.26	6.25 ± 0.53	71.65
5g	2.84 ± 0.36	6.50 ± 0.74	79.94
5h	2.78 ± 0.35	6.15 ± 0.32	75.19
5i	2.98 ± 0.38	6.00 ± 0.31	62.86
5j	2.80 ± 0.20	6.55 ± 0.37	83.07
5k	2.56 ± 0.31	6.90 ± 0.55	105.16
5l	2.80 ± 0.16	7.65 ± 0.28	107.44
5m	2.65 ± 0.15	6.44 ± 0.29	88.71
Diazepam	3.12 ± 0.22	8.15 ± 0.14	100.00

CNS, central nervous system.
[a]Each value represents the means ± SEM of six mice, significantly different from the control value at $p < 0.05$ (Student's *t*-test).

Table 4

Anticonvulsant activity ($n = 6$ animals, *orally*).

Compound	Dose (mg/kg)	% of animal protected against PTZ induced clonic convulsions
5d	9.0	33.33
	10.0	83.33
	11.0	100.00
5e	8.0	33.33
	9.0	66.66
	10.0	100.00
5k	5.0	16.66
	6.0	83.33
	7.0	100.00
5l	4.0	16.66
	5.0	66.66
	6.0	100.00
Diazepam	0.5	16.66
	1.0	50.00
	2.0	83.33
	2.5	100.00

CNS, central nervous system; PTZ, pentylenetetrazole.

Table 5

Effective dose (ED_{50}) protecting 50% population from pentylenetetrazole (85 mg/kg, orally) induced clonic convulsions ($n = 6$ animals) in mice.

Compound	ED_{50} mg/kg
5d	9.50
5e	8.50
5k	5.50
5l	4.50
Diazepam	1.00

with remaining substituted compounds. Present study explored that substitution of 1-chloromethyltriazole with pyrrolidine and piperidine moiety leads to the development of new chemical entities with potent CNS activity.

CONCLUSION

In the present investigation, 13 new triazolo[4,3-*a*] tetrahydrobenzo(*b*)thieno[3,2-*e*]pyrimidine-5(4*H*)-ones derivatives were synthesized and characterized by spectral data. They were screened for CNS depressant, skeletal muscle relaxant, and anticonvulsant activities; and compounds **5d**, **5e**, **5k**, and **5l** exhibited promising activities, which are comparable to the standard. The activity was attributed to the presence of propyl, isopropyl, pyrrolidinemethyl, and piperidinemethyl groups at position 1 on the condensed heterocyclic system containing tetrahydrobenzothiophenepyrimidine fused with triazole in the backbone structure of title compounds.

EXPERIMENTAL

Chemistry. All chemicals and solvents were supplied by Merck, S.D. Fine Chemical Limited, Mumbai. All the solvents were distilled and dried before use. The reactions were monitored with the help of thin-layer chromatography using pre-coated aluminum sheets with GF254 silica gel, 0.2 mm layer thickness (E. Merck). Melting points of the synthesized compounds were recorded on the Veego (VMP-MP) melting point apparatus. IR spectrum was acquired on a Shimadzu Infrared Spectrometer (model FTIR-8400S, Japan).^{1}H NMR spectra were recorded on a Bruker Avance II (400 MHz, Germany) spectrometer, and the chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as internal standard. Electron ionization mass spectra were recorded on a VG-250 spectrometer (VG Labs., Tritech England) with ionization energy maintained at 70 eV. Elemental analyses were obtained with an acceptable range (±0.4%) using a Perkin–Elmer 2400B CHN analyzer.

Synthesis of 2-hydroxy-3-(4-methylphenyl)-5, 6, 7, 8-tetrahydrobenzo (b) thieno-[2, 3-d] pyrimidin-4(3H)-one (2). Solution of bis (trichloromethyl) carbonate (29.6 g, 0.1 mol) in dichloroethane (300 mL) heated at 70°C then added dropwise solution of 4-methylphenylamine (10.7 g, 0.1 mol) in dichloroethane (100 mL) and reflux for 2 h. To the resultant solution of 4-methylphenylisocyanate in dichloroethane, a solution of 2-amino-3-carbethoxy-4,5,6,7-tetrahydrobenzo(*b*)thiophene **1** (22.5 g, 0.1 mol) in dichloroethane (100 mL) was added and refluxed for 3 h. Excess of solvent was removed under reduced pressure, and the crude residue of N^2-(3-carbethoxy)-N^1-(4-methylphenyl)-5,6,7,8-tetrahydrobenzo(*b*) thieno-2-yl urea was cyclized with potassium hydroxide solution (300 mL, 10%) for 3 h at reflux. The reaction mixture was cooled and filtered; and filtrate was acidified with dilute hydrochloric acid to yield compound (**2**). The separated solid was filtered, washed with water, dried, and recrystallized from ethanol. Yield: 22 g (71%); mp: 264–267°C; IR (KBr): 3220 (OH), 1680 (C═O) cm^{-1}; MS (*m*/*z*): 313 (M + 1).

Synthesis of 2-chloro-3-(4-methylphenyl)-5, 6, 7, 8-tetrahydrobenzo (b) thieno [2, 3-d] pyrimidin- 4(3H)-one (3). A mixture of 2-hydroxy-3-(4-methylphenyl)-5,6,7,8-tetrahydrobenzo(*b*)thieno-[2,3-*d*] pyrimidin-4(3*H*)-one **2** (10 g, 0.032 mol), phosphorus oxychloride (100 mL), and phosphorus pentachloride (5.0 g) was refluxed for 18 h at 90°C. Excess of phosphorus oxychloride was removed under reduced pressure. The residue obtained was dissolved in ethyl acetate, and organic layer was washed with saturated aqueous sodium bicarbonate solution and organic layer concentrated under reduced pressure. A crude compound obtained was purified by column chromatography through silica gel using a 20% ethyl acetate in n-hexane as an eluent to yield compound (**3**).

Yield: 6.2 g (59%); mp: 102–105°C; IR (KBr): 1680 (C═O), 723 (C–Cl) cm^{-1}; MS (*m*/*z*): 331 (M + 1).

Synthesis of 2-hydrazino-3-(4-methylphenyl)-5, 6, 7, 8-tetrahydrobenzo (b) thieno-[2, 3-d] pyrimidin-4(3H)-one (4). A mixture of 2-chloro-3-(4-methylphenyl)-5, 6, 7, 8-tetrahydrobenzo (*b*) thieno-[2, 3-*d*] pyrimidin-4(3*H*)-one **3** (5 g, 0.015 mol) and hydrazine hydrate (1.5 mL, 0.030 mol) in 50 mL of methanol was refluxed for 15 min and cooled to get white solid. The crude product was purified by recrystallization from ethanol. Yield: 3.4 g (69%); mp:197–200°C; IR (KBr): 3340, 3225 (NH_2), 1685 (C═O), 1600 (NH) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.70 (bs, 1H, NH), 7.40 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.24 (d, $J = 8.2$ Hz, 2H, Ar–H), 5.0 (bs, 2H, NH_2), 2.91–2.96 (t, $J = 6.0$ Hz, 2H, CH at C-8), 2.70–2.76 (m, 2H, CH_2 at C-5), 2.46 (s, 3H, CH_3), 1.74–1.86 (m, 4H, $2CH_2$ at C-6, C-7); MS (*m*/*z*):327 (M + 1).

Synthesis of 4-(4-methylphenyl)-6, 7, 8, 9-tetrahydro[1, 2, 4] triazolo[4,3-a]benzo(b)thieno[3,2-e]pyrimidine-5(4H)-one (5a). A mixture of *2*-hydrazino-3-(4-methylphenyl)-5, 6, 7, 8-tetrahydrobenzo (b) thieno-[2, 3-d] pyrimidin-4(3H)-one **4** (0.5 g, 0.0015 mol) and triethylorthoformate (10 mL) containing one drop of hydrochloric acid was refluxed for 5 h. The reaction mixture was cooled and separated solid was filtered, washed, dried, and recrystallized from ethanol. Yield: 0.41 g (68%); mp: 230–233°C; IR (KBr): 1690 (C═O) cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz, δ ppm): 8.41 (s, 1H, CH), 7.42 (d, $J = 8.3$ Hz, 2H, Ar–H), 7.25 (d, $J = 8.3$ Hz, 2H, Ar–H), 3.10 (t, 2H, $J = 6.1$ Hz, CH_2 at C-9), 2.79–2.81 (m, 2H,CH_2 at C-6), 2.44 (s, 3H, CH_3), 1.83–1.91 (m, 4H, $2CH_2$ at C-7, C-8); MS (*m*/*z*): 337 (M + 1); *Anal*. Calcd. for $C_{18}H_{16}N_4OS$: C, 64.27; H, 4.79; N, 16.65. Found: C, 64.22; H, 4.75; N, 16.68.

Synthesis of 4-(4-methylphenyl)-1-methyl-6,7,8,9-tetrahydro [1,2,4]triazolo[4,3-a]benzo(b)thieno[3, 2e]pyrimidine-5(4H)-one (5b). Prepared from compound **4** (0.5 g, 0.0015 mol) and triethylorthoacetate by using the same procedure as mentioned in

5a. Yield: 0.45 g (85%); mp: 276–279°C; IR (KBr): 1684 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.41 (d, $J = 8.3$ Hz, 2H, Ar–H), 7.31 (d, $J = 8.3$ Hz, 2H, Ar–H), 3.12 (t, $J = 6.0$ Hz, 2H, CH_2 at C-9), 3.01 (s, 3H, CH_3), 2.81–2.85 (m, 2H,CH_2 at C-6), 2.33 (s, 3H, CH_3), 1.87–1.91 (m, 4H, $2CH_2$ at C-7, C-8); MS (*m/z*): 351 (M + 1);*Anal*. Calcd. for $C_{19}H_{18}N_4OS$: C, 65.12; H, 5.18; N,15.99. Found: C, 65.10; H, 5.13; N, 15.97.

Synthesis of 1-ethyl-4-(4-methylphenyl)-6,7,8,9-tetrahydro [1,2,4]triazolo[4,3-a]benzo(b)thieno[3, 2-e]pyrimidine-5(4H)-one (5c). A mixture of compound **4** (0.5 g, 0.0015 mol) and propionic acid (10 mL) was refluxed for 10 h. The reaction mixture was poured on ice-water mixture and the separated solid was filtered, washed with water, dried, and recrystallized from ethanol. Yield: 0.30 g (55%); mp: 251–253°C; IR (KBr): 1675 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.53 (d, $J = 8.3$ Hz, 2H, Ar–H), 7.39 (d, $J = 8.3$ Hz, 2H, Ar–H), 2.97 (t, 2H, $J = 6.1$ Hz, CH_2 at C-9), 2.82–2.86 (m, 2H, CH_2 at C-6), 2.82 (q, 2H, CH_2), 2.35 (s, 3H, CH_3), 1.84–189 (m, 4H, $2CH_2$ at C-7, C-8), 1.39 (t, $J = 7.8$ Hz, 3H, CH_3); MS (*m/z*): 365 (M + 1); *Anal*. Calcd. for $C_{20}H_{20}N_4OS$: C, 65.91; H, 5.53; N, 15.37. Found: C, 65.88; H, 5.49; N, 15.35.

Synthesis of 4-(4-methylphenyl)-1-propyl-6,7,8,9-tetrahydro [1,2,4]triazolo[4,3-a]benzo(b)thieno[3,2-e]pyrimidine-5(4H)-one (5d). Prepared from the solution of compound **4** (0.5 g, 0.0015 mol) and butyric acid (10 mL) by using the same procedure as mentioned in **5c**. Yield: 0.35 g (61%); mp: 210–213°C; IR: 1680 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.51 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.35 (d, $J = 8.2$ Hz, 2H, Ar–H), 3.00 (t, $J = 6.0$ Hz, 2H, CH_2 at C-9), 2.80–2.84 (m, 2H,CH_2 at C-6), 2.58 (t, $J = 6.6$ Hz, 2H, CH_2), 2.37 (s, 3H, CH_3),1.82–1.87 (m, 4H, $2CH_2$ at C-7, C-8) 1.71 (m, 2H, CH_2), 1.10 (t, $J = 7.3$ Hz, 3H, CH_3); MS (*m/z*): 379 (M + 1); *Anal*. Calcd. for $C_{21}H_{22}N_4OS$: C, 66.64; H, 5.86; N, 14.80. Found: C, 66.64; H, 5.81; N, 14.79.

Synthesis of 1-isopropyl-4-(4-methylphenyl)-6,7,8,9-tetrahydro [1,2,4]triazolo[4,3-a]benzo(b)thieno[3,2-e]pyrimidine-5(4H)-one (5e). Prepared from compound **4** (0.5 g, 0.0015 mol) and isobutyric acid (10 mL) by using the same procedure as mentioned in **5c**. Yield: 0.40 g (67%); mp: 181–183°C; IR (KBr): 1685 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.55 (d, $J = 8.3$ Hz, 2H, Ar–H), 7.42 (d, $J = 8.3$ Hz, 2H, Ar–H), 3.21–3.33 (m, 1H, CH), 3.10 (t, $J = 6.2$ Hz, 2H, CH_2 at C-9), 2.83–2.87 (m, 2H,CH_2 at C-6), 2.43 (s, 3H, CH_3),1.81–1.86 (m, 4H, $2CH_2$ at C-7, C-8),1.23 (d, $J = 7.8$ Hz, 6H, CH_3); MS (*m/z*): 379 (M + 1); *Anal*. Calcd. for $C_{21}H_{22}N_4OS$: C, 66.64; H, 5.86; N, 14.80. Found: C, 66.60; H, 5.86; N, 14.78.

Synthesis of 1-mercapto-4-(4-methylphenyl)-6,7,8,9-tetrahydro [1,2,4]triazolo[4,3-a]benzo(b) thieno [3,2-e]pyrimidine-5(4H)-one (5f). To the solution of compound **4** (0.5 g, 0.0015 mol) in pyridine (10 mL) was added carbon disulfide (1 mL) at room temperature. The reaction mixture was refluxed for 2 h and allowed to cool to room temperature and poured on ice water. The solid separated was filtered, washed with water, dried, and recrystallized from ethanol. Yield: 0.46 g. (81%); mp: 279–281°C; IR (KBr): 1695 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.36 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.26 (d, $J = 8.2$ Hz, 2H, Ar–H) 4.67 (s, 1H, SH), 3.01 (t, $J = 6.1$ Hz, 2H, CH_2 at C-9), 2.80–2.85 (m, 2H, CH_2 at C-6), 2.47 (s, 3H, CH_3) 1.80–1.87 (m, 4H, $2CH_2$ at C-7, C-8); MS (*m/z*): 369 (M + 1); *Anal*. Calcd. for $C_{18}H_{16}N_4OS_2$: C, 58.67; H, 4.38; N,15.20. Found: C, 58.65; H, 4.35; N, 15.18.

Synthesis of 1-methylsulfanyl-4-(4-methylphenyl)-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]benzo(b) thieno[3,2-e]pyrimidine-5(4H)-one (5g). To the solution of compound **5f** (0.4 g, 0.001 mol) in methanol containing potassium hydroxide (0.06 g, 0.001 mol) was added dimethylsulfate (0.22 g, 0.001 mol) drop by drop with stirring. The reaction mixture was allowed to stand at room temperature for 10 h and poured onto ice water mixture. The solid separated was filtered, washed, and dried. It was recrystallized from ethanol. Yield: 0.35 g (85%); mp: 233–236°C; IR (KBr): 1682 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.41 (d, $J = 8.3$ Hz, 2H, Ar–H), 7.32 (d, $J = 8.3$ Hz, 2H, Ar–H), 3.14 (t, $J = 6.0$ Hz, 2H, CH_2 at C-9), 2.83–2.88 (m, 2H, CH_2 at C-6), 2.62 (s, 3H, SCH_3), 2.41 (s, 3H, CH_3), 1.81–1.88 (m, 4H, $2CH_2$ at C-7, C-8); MS (*m/z*): 383 (M + 1); *Anal*. Calcd. for $C_{19}H_{18}N_4OS_2$: C, 59.66; H, 4.74; N,14.65. Found: C, 59.65; H, 4.71; N, 14.64.

Synthesis of 4-(4-methylphenyl)-1-methylamino-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]benzo(b) thieno[3,2-e]pyrimidine-5(4H)-one (5h). To the solution of compound **4** (0.5 g, 0.0015 mol) in ethanol (20 mL) was added methyl isothiocyanate (0.11 g, 0.0015 mol). The mixture was refluxed on water bath for 6 h. Then it is cooled to room temperature, the separated solid was filtered, washed with ethanol, dried, and recrystallized from ethanol. Yield: 0.38 g (69%); mp: 153–156°C; IR (KBr): 3370 (NH), 1688 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.43 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.35 (d, $J = 8.2$ Hz, 2H, Ar–H), 5.21 (bs, 1H, NH), 3.81 (d, $J = 8.5$ Hz, 3H, CH_3), 3.10 (t, $J = 6.1$ Hz, 2H, CH_2 at C-9), 2.77–2.82 (m, 2H,CH_2 at C-6), 2.42 (s, 3H, CH_3), 1.84–1.89 (m, 4H, $2CH_2$ at C-7, C-8); MS (*m/z*): 366 (M + 1); *Anal*. Calcd. for $C_{19}H_{19}N_5OS$: C, 62.45; H, 5.24; N, 19.16. Found: C, 62.43; H, 5.25; N, 19.14.

Synthesis of 1-amino-4-(4-methylphenyl)-6,7,8,9-tetrahydro [1,2,4]triazolo[4,3-a]benzo(b)thieno[3,2-e]pyrimidine-5(4H)-one (5i). Mixture of compound **4** (0.5 g, 0.0015 mol) and cyanogen bromide (0.16 g, 0.0015 mol) in ethanol (20 mL) was refluxed for 5 h. The solid was filtered, washed with water, dried, and recrystallized from ethanol. Yield: 0.48 g (91%); mp: 270–273°C; IR (KBr): 3350, 3290 (NH), 1685 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.45 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.36 (d, $J = 8.2$ Hz, 2H, Ar–H), 4.40 (bs, 2H, NH_2), 3.14 (t, $J = 6.1$ Hz, 2H, CH_2 at C-9), 2.75–2.81 (m, 2H,CH_2 at C-6), 2.42 (s, 3H, CH_3), 1.89–1.95 (m, 4H, $2CH_2$ at C-7, C-8); MS (*m/z*): 352 (M + 1); *Anal*. Calcd. for $C_{18}H_{17}N_5OS$: C, 61.52; H, 4.88; N,19.93. Found: C, 61.50; H, 4.87; N, 19.92.

Synthesis of 1-chloromethyl-4-(4-methylphenyl)-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]benzo(b)thieno[3,2-e]pyrimidine-5 (4H)-one (5j). Mixture of compound **4** (1.0 g, 0.003 mol) and chloroacetyl chloride (0.346 g, 0.003 mol) in glacial acetic acid (10 mL) was refluxed for 10 h, and reaction mixture was poured on ice water mixture and the separated solid was filtered, washed with water, dried, and recrystallized from ethanol. Yield: 0.8 g (68%); mp: 178–181°C; IR (KBr): 1688 (C═O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.44 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.37 (d, $J = 8.2$ Hz, 2H, Ar–H), 5.24 (s, 2H, CH_2), 3.12 (t, $J = 6.2$ Hz, 2H, CH_2 at C-9), 2.72–2.78 (m, 2H,CH_2 at C-6), 2.42 (s, 3H, CH_3), 1.79–1.84 (m, 4H, $2CH_2$ at C-7, C-8); MS (*m/z*): 385 (M + 1); *Anal*. Calcd. for $C_{19}H_{17}ClN_4OS$: C, 59.29; H, 4.45; N, 14.56. Found: C, 59.28; H, 4.42; N, 14.51.

Synthesis of 4-(4-methylphenyl)-1-pyrrolidin-1-ylmethyl-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]benzo(b)thieno[3,2-e]pyrimidine-5 (4H)-one (5k). Mixture of 1-chloromethyl-4-(4-methylphenyl)-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]benzo(b)thieno[3, 2-e] pyrimidine-5(4H)-one **5j** (0.2 g 0.00052 mol), pyrrolidine (0.18 g,0.0025 mol), and anhydrous potassium carbonate (100 mg)

in dioxane (10 mL) was put in a round bottomed flask and refluxed for 12 h, cooled, and poured into ice water. The solid obtained was filtered, washed with water, dried, and recrystallized from ethanol. Yield: 0.12 g (57%); mp: 108–111°C; IR (KBr): 1680 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.48 (d, $J = 8.3$ Hz, 2H, Ar–H), 7.39 (d, $J = 8.3$ Hz, 2H, Ar–H), 3.78 (s, 2H, CH_2), 3.05 (t, $J = 6.2$ Hz, 2H, CH_2 at C-9), 2.74–2.80 (m, 2H,CH_2 at C-6), 2.41 (s, 3H, CH_3), 1.78–1.83 (m, 4H, $2CH_2$ at C-7, C-8), 1.41–1.61 (m, 4H,CH_2-pyrrolidinyl), 1.10–1.25 (m, 4H, CH_2-pyrrolidinyl); MS (*m/z*): 420 (M + 1); *Anal*. Calcd. for $C_{23}H_{25}N_5OS$: C, 65.85; H, 6.01; N, 16.69. Found: C, 65.83; H, 6.00; N, 16.68.

Synthesis of 4-(4-methylphenyl)-1-piperidin-1-ylmethyl-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]benzo(b)thieno[3,2-e]pyrimidine-5 (4H)-one (5l). Prepared from compound **5j** (0.2 g, 0.00052 mol) and piperidine (0.22 g, 0.0025 mol) by adapting same procedure as mentioned in **5k**. Yield: 0.10 g (45%); mp: 121–124°C; IR (KBr): 1679 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.34 (d, $J = 8.2$ Hz, 2H, Ar–H), 7.26 (d, $J = 8.2$ Hz, 2H, Ar–H), 3.84 (s, 2H, CH_2), 3.13 (t, $J = 6.2$ Hz, 2H, CH_2 at C-9), 2.72–2.78 (m, 2H,CH_2 at C-6), 2.34 (s, 3H, CH_3) 1.82–1.88 (m, 4H, $2CH_2$ at C-7, C-8), 1.41–1.63 (m, 4H, CH_2-piperidyl), 0.88–1.11 (m, 6H, CH_2-piperidyl); MS (*m/z*): 434 (M + 1); *Anal*. Calcd. for $C_{24}H_{27}N_5OS$: C, 66.49; H, 6.28; N, 16.15. Found: C, 66.45; H, 6.26; N, 16.18.

Synthesis of 4-(4-methylphenyl)-1-morpholin-4-ylmethyl-6,7,8,9-tetrahydro[1,2,4]triazolo[4,3-a]benzo(b)thieno[3,2-e]pyrimidine-5 (4H)-one (5m). Prepared from compound **5j** (0.2 g, 0.00052 mol) and morpholine (0.21 g, 0.0025 mol) by adapting same procedure as mentioned in **5k**. Yield: 0.14 g (64%); mp: 141–144°C; IR (KBr): 1691 (C=O) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz, δ ppm): 7.32 (d, $J = 8.3$ Hz, 2H, Ar–H), 7.22 (d, $J = 8.3$ Hz, 2H, Ar–H), 3.82 (s, 2H, CH_2), 2.94 (t, 2H, $J = 6.1$ Hz, CH_2 at C-9), 2.71–2.77 (m, 2H,CH_2 at C-6), 2.33 (s, 3H, CH_3),1.82–1.89 (m, 4H, $2CH_2$ at C-7, C-8), 1.70–1.75 (m, 4H,CH_2-morpholinyl), 1.20–1.41 (m, 4H, CH_2-morpholinyl); MS (*m/z*): 436 (M + 1); *Anal*. Calcd. for $C_{23}H_{25}N_5O_2S$: C, 63.43; H, 5.79; N, 16.08. Found: C, 63.44; H, 5.77; N, 16.00.

PHARMACOLOGICAL SCREENING

CNS depressant activity by photoactometer method. The title compounds (**5a–m**) were screened for their CNS depressant activity using photoactometer [29,30] at 30 min and 1 h after drug administration. The CNS depressant activity of animal inside the photometer chamber was recorded as a photoactometer counts. Decreased score suggests the CNS depressant activity. The Swiss albino mice of either sex weighing 25–30 g were used. They were divided into groups of six animals each and each group was allowed to get acquainted for 10 min. Thereafter, the photoactometer counts were noted for a period of 10 min, which was the initial reading (control). The test compounds were suspended in 1% CMC solution in distilled water and administered at a dose of 5 mg/kg i.p. of the body weight. Each group is served as its own control. One of the groups was treated with diazepam as the standard at a dose of 5 mg/kg. After 20 min of administration of test compound, the animals were kept into the photoactometer chamber, and the counts were noted for 10 min after a 10 min rest in the chamber. The same procedure was repeated after 50 min. Decrease in the number of counts for each group was calculated, and the % CNS depressant activity was determined by the following formula.

$$\%\ \text{CNS depressant activity} = (\text{control reading} - \text{in count}/\text{control reading}) \times 100$$

The observations are tabulated in Table 1.

CNS depressant activity by forced swim pool method [31]. CNS depressant activity of test compounds (**5a–m**) was measured in Albino mice using Forced Swimming Test. Albino mice were placed in chamber (diameter 45 cm, height 20 cm) containing water up to a height of 15 cm at 25 ± 2°C for 15 min. At 5 to 6 min later, immobility reached a plateau, where the mice remained immobile for approximately 80% of the time. After 15 min in the water, the mice were removed and allowed to dry in a heated enclosure before being returned to their home cages. They were again placed in the chamber after 24 h, and animals were administrated (5 mg/kg) the test compound i.p. 30 min before the test session. The period of immobility (passive floating without struggling, making only those movements which are necessary to keep its head above the surface of water) during the 5-min test period was measured. The increase in immobility period indicates that the compound exhibited CNS depressant activity. The results are presented in Table 2.

Skeletal muscle relaxant activity (Motor coordination) by rotarod method [32]. Motor coordination was tested using rotarod apparatus. The mice weighing 25–30 g were divided into group of six animals each. The mice were trained to stay on an accelerating rotarod that rotated at 6 rpm. The test compounds were suspended in 1% CMC solution in distilled water and administered at a dose of 5 mg/kg i.p. of the body weight. Each group is served as its control. One of the groups was treated with diazepam as the standard at a dose of 5 mg/kg. After 30 min of administration of test compound, the animals were placed on the rotarod apparatus and again the number of falls per min was recorded. Mean increase in number of falls per minute for each group was calculated and skeletal muscle relaxation activity was determined by following the formula.

$$\%\ \text{Muscle relaxant relaxant activity} = (\text{Mean reading in no. of falls} - \text{Mean control reading}/\text{Mean control reading}) \times 100$$

The observations are tabulated in Table 3.

Anticonvulsant activity (PTZ induced seizure test) [33]. Of the various compounds tested for CNS depressant activity, the most active four derivatives **5d**, **5e**, **5k** and **5l** were evaluated for anticonvulsant activity. The dose required for

protecting the animal from PTZ induced clonic convulsions was determined for tested compound (Table 4). Aqueous solution of PTZ (dose 85 mg/kg) was administered orally to group of six mice, 30 min after the administration of the test compound as suspension in 1% CMC i.p. Mice were then observed for a period of 20 min for the symptoms of clonic convulsions. Number of animals in each group, which were protected against PTZ induced clonic convulsions, was used as a percentage response parameter for calculating the effective anticonvulsant dose (ED_{50}) of the test compound.

All the tested compounds offered good protection to the animals from the PTZ induced clonic convulsions at the dose levels 6.0–12.0 mg/kg, body weight. The compound **5l** exhibited the best anticonvulsant activity by protecting 100% animals at dose of 6.0 mg/kg body weight. ED_{50} of these compounds have also been calculated and tabulated in Table 5.

Statistical analysis. Data obtained for each set of CNS depressant activity were expressed as mean change in photoactometer count ± SEM, and data from muscle relaxant activity by rotarod method were expressed as mean fall of time ± SEM, and both activities were analyzed by one way analysis of variance followed by Student's *t*-test. All statistical calculations were performed using evaluation version of Graph Pad Prism 3.0 (USA) statistical software.

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